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Patent Docket P1243R1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Deborah Ann Ansaldi Serial No.: 09/320,100 Filed: 26 MAY 1999 For: SEPARATION OF POLYPEPTIDE MONOMERS	Group Art Unit: 1642 Examiner: J. Hunt

CERTIFICATE OF MAILING
I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on

September 11, 2001

Pamela Gavette

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DECLARATION UNDER 37 CFR §1.132

Assistant Commissioner of Patents
Washington, D.C. 20231

I, Steven M. Cramer, PhD, do hereby declare and say as follows:

considered 12-28-01

1. I hold a B.S. in Biomedical Engineering from Brown University, a M.S. in Chemical Engineering from Yale University, and a Ph.D. in Chemical Engineering from Yale University. From 1978 to 1981 I worked as an engineer at Amicon Corporation, and from 1986 to the present I have held a teaching position at Rensselaer Polytech Institute (RPI). I am at present Professor of the Department of Chemical Engineering at RPI. I am being compensated for this Declaration.

2. My educational background, professional experience, professional activities, research and equipment grants, patents, honors and awards, and list of publications are provided in Appendix A attached to this Declaration.

3. I am intimately familiar with chromatographic separation of proteins and have employed such methodology in my scientific studies since 1981 as described in Appendix A. Specifically, since coming to RPI in 1986, I have become a recognized expert in the fields of chromatographic bioprocessing and separation

science. I am a co-inventor of low-molar-mass displacer technology for protein purification, and have developed several mathematical models of protein chromatography that allow accurate prediction of effluent profiles in process-scale separations. In addition, I have extensive experience in membrane separations, enzyme technology, and environmental separations. Also, I am the editor of the journal Separation Science and Technology and am a fellow of the American Institute for Medical and Biological Engineering.

4. I have reviewed the above-identified application, the Office Action after Final Rejection dated 10/12/00, the Preliminary Amendment filed together with a continued prosecution application of the above application, the latest Office Action dated 5/22/01, and the cited references, Yang *et al.*, Journal of Chromatography, A 743 (1996), Hahn *et al.*, Chromatography, 795, 277-287 (1998), US 4,764,279 (Tayot *et al.*), and Oncogene Science catalog 1992, pages 18 and 34. It appears that the Examiner is basing the rejections on two key assumptions: 1) the protein mixtures to be eluted in the cited references Yang *et al.*, Hahn *et al.*, and Tayot *et al.* contain dimers and multimers of the protein whose separation is desired, and 2) whether or not (1) is true, if a protein is purified from any protein mixture using ion-exchange chromatography by methods set forth in the claims of the above-identified application, this will inherently lead to the separation of the monomers from their own dimers/multimers at high yield and purity. In my considered opinion after review of all the above documents, neither of these assumptions is true. The basis for my opinion and the reasons why I believe the claimed invention is patentable over the cited references follow.

5. The claimed invention is directed to a method for separating polypeptide monomers from a mixture comprising said polypeptide monomers, and dimers or multimers of said polypeptide monomers or both dimers and multimers of said polypeptide monomers, wherein the method consists essentially of applying the mixture to a cation-exchange or anion-exchange chromatography resin in a buffer, wherein if the resin is cation-exchange, the pH of the buffer is about 4-7, and wherein if the resin is anion-exchange, the pH of the buffer is about 6-9, and eluting the mixture at a gradient of about 0-1 M of an elution salt. The method necessarily results in the separation of the monomer from its dimers and/or multimers present in the

mixture and to such a degree that the monomer has a purity of greater than 99.5% and the monomer yield is greater than 90%.

6. At the relevant time of filing the above application (June 1, 1998), the disclosure of Yang *et al.* would not have conveyed to the skilled practitioner in this field the above stated result, i.e., that monomeric proteins can be purified from dimeric and/or multimeric forms thereof and obtained in a yield of such high degree utilizing the ion-exchange technique of Yang *et al.* One skilled in the chromatographic field would view Yang *et al.* in the context in which it is written. Ion-exchange chromatography is a common method for separating proteins, and Yang *et al.* merely utilizes this technique to carry out what would be expected in the art. Thus, Yang *et al.* are separating polypeptide monomers from other monomeric forms thereof (such as differently glycosylated or post-translationally different immunoglobulins), or from totally different polypeptide monomers contained in the ascites and sera, or from dimers and/or multimers that may be naturally contained in ascites and sera. However, Yang *et al.* do not explicitly disclose separation of such monomers from <u>their own</u> dimers and/or multimers. The skilled artisan would not have believed as of June 1, 1998 that separation of monomers from their own dimers and/or multimers to produce therapeutically acceptable polypeptides could be accomplished at such high yield and purity by ion-exchange chromatography. Before the filing date of this application, the skilled chromatographic separation scientist was using size-exclusion chromatography for this purpose. Yang *et al.* do not tell the skilled scientist that ion-exchange chromatography is the answer, since ascites and sera loaded onto the column are complex mixtures of components that do not necessarily contain such dimers and/or multimers. Thus, in my view one of reasonable skill in the field would not believe that Yang *et al.* discloses all stated features and elements of the claimed invention.

7. The protein loads utilized by Yang *et al.* in their chromatography would not allow one of reasonable skill in this field to reach the conclusion that purification of monomers from their dimers and/or multimers would be feasible, much less would necessarily flow from the disclosure of Yang *et al.*

8. Moreover, one of ordinary skill in the field as of June 1998 would not have appreciated or recognized from Yang *et al.* the feature thought to be inherent, namely, that dimers and

multimers could be separated from their own monomers, let alone the minimum yields or purity levels stated. As mentioned above, there are chromatographic media designed specifically to separate proteins by size (size-exclusion chromatography) and these were used by practitioners before June 1998 to achieve the separation of monomers from their dimers and/or multimers as claimed. The practitioner versed in this field would not have recognized that monomers could be separated to the level of purity and yields claimed; evidence to the contrary is shown by the fact that such ion-exchange purification methods were not used to purify monomers from their dimers and/or multimers before the present invention was made, but rather size-exclusion chromatography. Since Yang *et al.* do not contain the supporting data or text describing separation of monomers from their dimers and/or multimers, the skilled practitioner, without the teachings disclosed for the first time by the present application, would not have recognized that separation of monomers from their dimers and/or multimers at such high yields and purity would be possible using the claimed method of purification.

9. Similarly, all of the elements and features of the claimed invention are not disclosed by Hahn *et al.* since the claims require that the monomer be separated from its own dimers and/or multimers. Hahn *et al.* teach separation of various different proteins from each other, all of which are contained in bovine whey, such as IgG from lactoferrin and from lactoperoxidase (see, e.g., Table 1 on page 280). There is no evidence in Hahn *et al.* that any separation has occurred between the monomer and any of its own dimers and/or multimers present in the mixture, as required by the present claims, as opposed to dimers and/or multimers that may naturally be present in bovine whey.

10. The Examiner states that Hahn *et al.* teach purification of immunoglobulins using an identical method to that instantly claimed and thus purification would include elution of the IgG monomers from a mixture (bovine whey) which contains monomers and dimers or multimers. However, as established above, the separation of monomers from dimers and/or multimers thereof (the characteristic of the claimed invention deemed to be inherent) is not necessarily or actually achieved by practicing the ion-exchange technique with the protein load mixture used by Hahn *et al.* to purify immunoglobulins from bovine whey. The ordinarily skilled scientist versed in purification techniques would not

have reasonably concluded from the teachings of Hahn *et al.* that purification of monomers from their dimers and/or multimers would be feasible in June 1998.

11. Further, the skilled scientist in chromatography would not have appreciated or recognized as of June 1998 from Hahn *et al.* that monomers could be separated from their dimers and/or multimers. Since Hahn *et al.* do not contain the requisite teachings, the skilled scientist would not have recognized that separation of monomers from their dimers and/or multimers would be possible using the claimed method of purification.

12. As to levels of purity, Hahn *et al.* obtain mediocre results upon purification of IgG from other whey proteins (not from its dimers/multimers). In all lanes of the SDS-PAGE gels of Fig. 4 IgG co-elutes with at least one of the other whey proteins. One skilled in this field would conclude from this figure that the separations were not optimal for the purification of IgG from whey proteins, and none achieved close to the 99.5% purity shown in the Examples of the above application, which reflects the separation level of monomer from its own dimers and/or multimers, not just from whey proteins. The authors of Hahn *et al.* include comments such as "When IgG is eluted from S-Sepharose FF, it co-elutes with beta-lactoglobulin and alpha-lactalbumin (page 287, par. 2)" and, "A second purification step must be added, if high purity of a certain protein is desired (p. 287, par. 3)," indicating that the method does not achieve optimal purification of any protein, much less a polypeptide monomer from its dimers and/or multimers.

13. The claimed invention would also not have been obvious as of June 1998 from the combination of Tayot *et al.* with Yang *et al.* Tayot *et al.* does not disclose or suggest how one skilled in the art might separate proteins from their own dimers and/or multimers. Instead, hemoglobin, gamma-globulins, and albumin are separated from each other and presumably also from other unrelated proteins in the blood (see, e.g., claim 1), or hemoglobin and albumin are separated from each other and presumably also from other unrelated proteins in the blood (see, e.g., claim 10). These protein moieties are not related as monomers and dimers of such monomers and/or multimers of such monomers, as is required in the claimed method of the above application. The anion-exchange step described in Tayot *et al.* is designed such that only albumin binds to the column and the hemoglobin and immunoglobulins flow through the column. The

gamma-globulins are separated from the hemoglobin by precipitation in ice-cold ethanol. Therefore, the purification of IgGs is achieved by a method (precipitation) completely distinct from the present claimed ion-exchange method (see col. 4, lines 10-54 of Tayot *et al.*). Tayot *et al.* further state that the Ig precipitate "...must then be subjected to other purification operations already known so as to prepare immunoglobulins which may be used in human therapeutics" (col. 4, lines 48-51), just as with albumin (compare col. 4, lines 29-31). It is evident that the anion-exchange method described does not purify the gammaglobulins or albumin from its dimers and/or multimers. These statements regarding further purification that is required actually teach away from the claims of the above application where no further purification step is used.

14. The claimed invention also would not have been obvious from the Oncogene Science catalog along with Yang *et al.* and/or Hahn *et al.* The latter references contained no details or directions to instruct the skilled artisan on how to obtain pure antibodies from impure mixtures containing dimers and multimers of the antibody monomers to be separated for the reasons noted above. Further, the so-called highly purified antibodies of the catalog are actually only research-grade material, so that their level of purity has no bearing on the level of purity needed to obtain antibodies suitable for therapeutic needs, as the claimed level of greater than 99.5% reflects. Such antibodies must be much more highly purified. In fact, neither Yang *et al.* nor Hahn *et al.* nor the Oncogene Science catalog even acknowledges the existence of dimers and/or multimers of polypeptide monomers, let alone that a separation thereof from the monomers can occur so as to obtain highly pure monomeric antibodies. The combined references would not have suggested the claimed invention as set forth above, particularly with the purity and yield results.

15. Furthermore, when I first heard about the invention claimed in the above application, I was surprised that the technique could be used to separate monomers from their own dimers and/or multimers at such unexpectedly high minimum purity and yield levels obtained as claimed, i.e., greater than 99.5% and greater than 90%, respectively. Size-exclusion chromatography was the gold standard at the time for distinguishing between these very similar protein species. My colleagues and I working in the separation arts would not have

expected from Yang *et al.* combined with Tayot *et al.* or from Yang *et al.* and Hahn *et al.* in combination with the selected catalog pages that such a high yield and purity could be achieved.

16. In summary, the above citations alone or in combination merely disclose that proteins can be purified to some degree using ion-exchange chromatography. In particular, the disclosures clearly show separation of IgG from BSA or IgG partially separated from whey, serum, or ascites proteins, etc. None of the cited references even mentions the existence of dimers and/or multimers of polypeptide monomers. Nowhere do these references, alone or in combination, mention or suggest the separation of monomers from their dimers/multimers using ion-exchange chromatography as claimed, much less with the claimed yield and purity results. Such results would not have necessarily followed from practicing the teachings of these references due to the nature of the mixtures being loaded on the column in these references, and the skilled practitioner would not have appreciated or expected from these teachings that such could be done.

17. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

9/6/01
Date

Steven M. Cramer, Ph.D.

APPENDIX A

STEVEN M. CRAMER

Home Address
783 Trottingham Drive
Schenectady, NY 12309
(518) 377-9074

Business Address
Department of Chemical Engineering
Rensselaer Polytechnic Institute
Troy, NY 12180-3590
(518) 276-6198

Birthdate: 2/11/56

EDUCATIONAL BACKGROUND

1986 PhD in Chemical Engineering, Yale University, New Haven, Connecticut

1982 Master of Science in Chemical Engineering, Yale University, New Haven, Connecticut

1978 Bachelor of Science in Biomedical Engineering, Brown University, Providence, Rhode Island

PROFESSIONAL EXPERIENCE

5/95-Present: Professor of Chemical Engineering, Department of Chemical Engineering, Rensselaer Polytechnic Institute, Troy, New York. Conducting research on: Novel Low Molecular Weight Displacers for Protein Purification in Ion Exchange Chromatographic Systems, Optimization of Preparative Ion Exchange Chromatography, Purification of Proteins from Complex Biological Mixtures by Displacement Chromatography, Theoretical and Experimental Studies in Metal Affinity Chromatography, Molecular Modeling of Displacer-Surface Interactions, Ultrapurification of Isopropyl Alcohol for Semiconductor Applications, Recycling of Metal Plating Baths from Metal Plating Operations. Teaching courses in: material and energy balances, separation processes, and chromatographic separation processes.

7/93-5/95: Isermann Associate Professor of Chemical Engineering, Department of Chemical Engineering, Rensselaer Polytechnic Institute, Troy, New York.

1/90-7/93: Associate Professor of Chemical Engineering, Department of Chemical Engineering, Rensselaer Polytechnic Institute, Troy, New York.

9/86 - 1/90: Isermann Assistant Professor of Chemical Engineering, Department of Chemical Engineering, Rensselaer Polytechnic Institute, Troy, New York.

9/78 - 8/81: Research Engineer, Amicon Corporation, Lexington, Massachusetts.

HONORS

7/99 Chair of Gordon Conference on Reactive Polymers and Ion Exchange
12/96 Elected a Fellow of American Institute for Medical and Biological Engineering
3/96 Executive Editor: Separation Science and Technology
5/95 Special Associate Editor: Biotechnology and Bioengineering

5/90	Early Career Award, Rensselaer Polytechnic Institute.
3/89	Presidential Young Investigator, National Science Foundation.
3/89	Lilly Teaching Fellow Award, Rensselaer Polytechnic Institute.
9/87	Dow Chemical Company Excellence in Teaching Award.
9/86-Present	Editorial Board, Isolation and Purification

PROFESSIONAL ACTIVITIES

Membership in: American Chemical Society, American Institute of Chemical Engineers, American Association for the Advancement of Science.

Co-Chair Biotechnology Secretariat Program for 1994 San Diego ACS Meeting.
Executive Committee, Biotechnology Secretariat, American Chemical Society.
Executive Committee, I & EC Division, American Chemical Society.
Executive Committee, Separation Science and Technology Subdivision, American Chemical Society.

Reviewer for the following journals and agencies

National Science Foundation
Journal of Chromatography
Biotechnology Journal
Reactive Polymers
Biotechnology and Bioengineering
I&EC Research
Preparative Chromatography
Chemical Engineering Science
Proceedings of National Academy of Sciences
M.I.T. (Ph.D. Thesis)
Health Effects Institute
Nature
Trends in Analytical Chemistry
Chemical Engineering Communications
Biotechnology Progress
AIChE Journal
University of Queensland (PhD Thesis)
Yale University (PhD Thesis)
American Scientist
Separations Technology
Chemistry of Materials
Biotechnology Advances
National Institute of Health
Computers in Chemical Engineering
Journal of Colloid and Interface Science

Involved in following activities at Rensselaer

Bioseparations and Biophysics Centers, Chair of Chemical Engineering Curriculum Committee, Engineering School Committee on Reward Structure.

Consulting

Consulting: Served as a consultant to Millipore Corporation, H. R. Parrs Associates, Boehringer Labs, NASA, MARS Corporation, Allied Signal, National Institute of Health, and Health Effects Institute, Merck, Protein Design Labs, Pharmacia, Chiral Technologies, Genentech, IGEN, DYAX, Regeneron.

Sessions Chaired at Meetings

Novel Bioseparations Processes, ACS 96 Spring Meeting, New Orleans, LA. (1996)

Chromatographic Bioprocessing, Recovery of Biological Products VIII, Tucson, Arizona. (1996)

Preparative Chromatography, Prep 95, Washington, DC. (1995)

Mass Transport in Chromatographic Systems, International Society of Proteins, Polynucleotides, and Peptides, Boston, MA. (1995)

Biotechnology, Pacifichem, Hawaii. (1995)

Bioseparations, ACS Meeting, San Diego, CA (1994).

Protein Separations, Gordon Conference on Separation and Purification, New London, NH (1994).

Bioseparations, ACS Meeting, Denver, CO (1993).

Bioseparations, AIChE Meeting, St. Louis, MO (1993).

Sessions Co-Chairman, "Transport Processes in Bioseparations Systems (I and II), AIChE Annual Meeting, Miami, FL (1992).

Session co-chairman, "Chromatographic Engineering in Bioseparations", AIChE Annual Meeting, Los Angeles, CA, 1991.

Session chairman, "Mathematical Models of Preparative Chromatography", 8th International Symposium on Preparative Chromatography", Arlington, VA, 1991.

Session co-chairman, "Novel Engineering Approaches to Bioseparations", ACS National Meeting, Washington, D.C., 1990.

Session chairman, "Chromatography", Gordon Conference on Separations and Purifications, New London, NH, 1990.

Session co-chairman. "Transport Processes in Bioseparation Systems", AIChE Annual Meeting, San Fransisco, 1989.

Session co-chairman. "Young Faculty Forum", AIChE Annual Meeting, Washington, 1988.

Session co-chairman. "Transport Processes in Bioseparation Systems" AIChE Annual Meeting, Washington, 1988.

RESEARCH GRANTS

"Purification of Complex Biological Mixtures by Displacement Chromatography", Regeneron Pharmaceuticals, funding period 5/96-6/99, grant award $100,000.

Optimization of Preparative Ion Exchange Chromatography, PI Dr. Steven Cramer and Dr. Wayne Bequette, funding period 10/1/98-10/1/00, grant award $160,000.

Hazardous Source and Waste Reduction in Metals Finishing Industry by Hybrid Membrane Separation Systems, NYSERDA, Co-P.I.'s, S. Cramer and W. Gill, funding period 1/95 - 1/99, grant award $269,000.

Purification of Complex Biological Mixtures by Displacement Chromatography, Lederle Praxis., grant award $30,000.

Reversed Phase Purification of Antibiotics, Pfizer, funding period 1/98-12/99, grant award $24,251

Hydrophobic Displacement Chromatography of Proteins, NSF, PI Dr. Steven Cramer, funding period 1/99-1/02, grant award $240,000. (Recommended for funding, waiting for final approval).

Purification of Oligonucleotides, ISIS Pharmaceuticals, $40,000.

Hydrophobic Displacement Chromatography, Biogen, $81,375.

Optimal Design of Stationary Phase Materials, Pharmacia, $30,000.

Low Molecular Weight Displacers for Protein and Oligonucleotide Purification, National Institute of Health, Co-P.I.'s Dr. Steven Cramer and Dr. James Moore, funding period 12/1/1999 -1/03, $641,934.

"Purification of Complex Biological Mixtures by Displacement Chromatography", Regeneron Pharmaceuticals, funding period 5/96-6/97, grant award $50,000.

"Reproducibility of Preparative Ion Exchange Materials", Pharmacia, funding period 5/96-6/97, grant award $15,000.

Optimization of Preparative Ion Exchange Chromatography, PI Dr. Steven Cramer (consultant Dr. Wayne Bequette), funding period 8/15/95-8/15/98, grant award $189,473.

Engineering Research Equipment: An Infrared Spectrometer for the Analysis of Protein - Surface Interactions, Co PI's T. M. Przybycien, G. Belfort, and S. M. Cramer, grant award $32,000.

Low Molecular Weight Displacers for Protein Purification, National Institute of Health, Co-P.I.'s Dr. Steven Cramer and Dr. James Moore, funding period 9/1/95 - 9/1/98, grant award $557,898.

Hazardous Source and Waste Reduction in Metals Finishing Industry by Hybrid Membrane Separation Systems, NYSERDA, Co-P.I.'s, S. Cramer and W. Gill, funding period 1/95 - 1/97, grant award $219,000.

Low Molecular Weight Displacers for Protein Purification, National Science Foundation, CO-P.I.'s Dr. Steven Cramer and Dr. James Moore, funding period 10/1/94 - 10/1/95, grant award $124,650.

"Novel Supports for Displacement Chromatography", Pharmacia AS, P.I., Dr. Steven Cramer, funding period, 4/93 - 8/95, grant award $54,350.

"Chromatographic Purification of Isopropyl Alcohol", NYSERDA, Co-P.I.'s, S. Cramer and W. Gill, funding period 2/1/94 - 6/1/95, grant award $100,000.

Presidential Young Investigator, National Science Foundation, funding period 10/1/89 - 10/1/94, total grant award $312,500.

"Optimization and Scale-Up of Displacement Chromatography", Millipore Corporation, P.I., Dr. Steven M. Cramer, funding period 9/1/89 - 9/1/94, grant award $120,000.

"Displacement Chromatography of Proteins: Development of Pentaerythritol-Based Displacers", National Science Foundation, CO-P.I.'s Dr. Steven Cramer and Dr. James Moore, funding period 9/91 - 9/93, grant award $160,000.

"Purification of IgG's from Whey", Immucell Corp., P.I., Dr. Steven Cramer, funding period 1/93 - 1/94, grant award $8,000.

"Preparative-Scale Separation of Biopolymers by Displacement Chromatography", National Science Foundation, P.I., Dr. Steven M. Cramer, funding period, 6/1/87 - 10/30/89, grant award $70,000.

"Lilly Teaching Fellowship", funding period 7/1/89 - 7/1/90, grant award, $6,500.

"Dow Chemical Company Excellence in Teaching Award", grant award, $10,000.

"Multicomponent Adsorption Behavior of Biopolymers on Chromatograhic Surfaces", The Petroleum Research Fund, American Chemical Society, P.I., Dr. Steven M. Cramer, funding period 1/88 - 1/90, grant award $18,000.

"Identification and Isolation of Cyclosporine Metabolites Associated with Nephrotoxicity and Immunosuppression", with Albany Medical College, National Institute of Health, funding period 7/88 - 7/91, total grant award $390,000. Dr. Cramer's award, $100,000.

"Purification of Complex Biological Mixtures by Displacement Chromatography: Extension to Group Specific Affinity Supports", National Science Foundation, P.I. Dr. Steven M. Cramer, funding period, 2/1/90 - 7/31/92, grant award $60,000.

"Purification of Proteins", Eastman Kodak, P.I., Dr. Steven M. Cramer, funding period 9/1/89 - 9/1/92, grant award $30,000.

"Downstream Bioprocessing with Novel Membrane Systems", Hoechst Celanese, P.I. Dr. Steven M. Cramer, funding period 1/90 - 9/92, grant award $20,000.

"Purification of Therapeutic Proteins using Group Specific Affinity Displacement Chroma-tography", Abott Biotech, Inc., P.I. Dr. Steven Cramer, funding period 9/91 - 9/92, grant award $28,000.

EQUIPMENT GRANTS

Diode Array Detector, Protein Design Labs
Autoprep 500 Preparative Chromatographic System, Millipore Corporation.
Deltaprep Preparative Chromatographic System, Millipore Corporation.
FPLC Preparative Chromatographic System, Pharmacia-LKB Corporation.
Automated Electrophoresis System, Pharmacia - LKB Corporation.

Patents

US Patent # 5,478,924 " Displacement Chromatography of Proteins using Low Molecular Weight Displacers", S. M. Cramer, J. A. Moore, A. Kundu, Y. Li, G. Jayaraman.

PUBLICATIONS

Refereed Journal Articles

A. Kalra, N. Tugcu, S. M. Cramer, and S. Garde, "Salting-In and Salting-Out of Hydrophobic Solutes in Aqueous Salt Solutions", J. Phys. Chem. B, 105 (27), 6380 -6386, 2001.

Shukla, A., Deshmukh, R., Moore, J. and Cramer, S.M., "Purification of Oligonucleotides by High Affinity, Low Molecular Weight Displacers", BIOTECHNOL PROGR, 16 (6): 1064-1070 NOV-DEC 2000

S. Ghose and S. M. Cramer, "Characteriation and modeling of monolithic stationary phases: applications to preparative chromatography", in press, J. Chromatogr.

Shukla, K.M. Sunasara, R. G. Rupp and S. M. Cramer, "Hydrophobic displacement chromatography of proteins", Biotech and Bioeng, 68: (6) 672-680 (2000).

Natarajan V., Bequette B.W., and Cramer, S.M. "Optimization of ion exchange displacement separations. I. Validation of an iterative scheme and its use as a methods development tool", J CHROMATOGR A, 876: (1-2) 51-62 APR 21 2000

Natarajan V. and Cramer, S.M. "Optimization of ion exchange displacement separations. II. Comparison of displacement separation on various ion exchange resins", J CHROMATOGR A, 876: (1-2) 63-73 APR 21 2000

Natarajan, V., and Cramer, S. M., "A Methodology for the Characterization of Ion-Exchange Resins", SEPAR SCI TECHNOL, 35: (11) 1719-1742 2000.

Shukla, A., and Cramer, S. M., "Bioseparations by Displacement Chromatography", in the Handbook of Bioseparations, Academic Press. 2000.

Tugcu, N.,Mazza, C.,Breneman, C., Sanghv, Y.and Cramer, S.M.High Throughput Screening and Quantitative Structure Efficacy Relationship Models for Designing Displacers for Antisense Oligonucleotide Purification in Anion-Exchange Systems, submitted to Sep. Sci. and Tech.

Tugcu, N., Deshmukh, R., Sanghvi, S., Moore, J. and Cramer, S.M., "Purification of Oligonucleotides at High Column Loading by High Affinity, Low Molecular Weight Displacers, J CHROMATOGR A, 923 (1-2): 65-73 JUL 20 2001

Mazza, C, Cramer, S M; "Evaluation of lot-to-lot consistency in ion exchange chromatography" J LIQ CHROMATOGR RELAT TECHNO, 22: (11) 1733-1758 1999

Natarajan, V., and Cramer, S. M., "Modeling Shock Layers in Ion Exchange Displacement Chromatography", AIChE J. 45 (1999) 27-37.

Sane, S., Cramer, S. M., and Przybycien, T. M., "A Holistic approach to Protein Secondary Structure Characterization Using Amide I Band Raman Spectroscopy", Analytical Biochemistry 269, (1999) 255-272.

Sane, S., Cramer, S. M., and Rpzybycien, T M., "Protein structure perturbations on chromatographic surfaces", Journal of Chromatography A 849 (1999) 149-159.

Cramer, S. M., Natarajan, V., "Chromatography, Ion Exchange" in the Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis, and Bioseparation, (1999) 612-627.

Barnthouse, K. A., Trompeter, W., Jones, R., and Cramer, S. M., "Ion-exchange displacemenmt chromatography: Scale-up and displacer clearance for recombinant human brain-derived neurotrophic factor (rHuBDNF)", BioPharm (1999) 35-44.

Sunasara, K.M., Cramer, S.M., Hauer, C.R., Rupp, R.G., and Shoup. V.A. (1999). Characterization of Recombinant Human Brain-Derived Neurotrophic Factor Variants. Archives of Biochemistry and Biophysics Vol 372, No. 2, pp248-260.

Natarajan, V., and Cramer, S. M., "Modeling Shock Layers in Ion Exchange Displacement Chromatography", AIChE J. 45 (1999) 27-37.

Barnthouse, KA; Trompeter, W; Jones, R,Rupp, R. and Cramer, S.M. Ion-exchange displacement chromatography: Scale-up and displacer clearance for recombinant human brain-derived neurotrophic factor LC GC-MAG SEPARATION SCI, 17: (11) 1028-+ NOV 1999

S. Vunnum, V. Natarajan, and S. M. Cramer "Non-Linear Multicomponent Gradient Chromatography in IMAC Systems", Sep. Sci. and Tech. 33: (16) 2465-2489 (1998)

Shukla, AA; Bae, SS; Moore, JA, et al. "Synthesis and characterization of high-affinity, low molecular weight displacers for cation-exchange chromatography", IND ENG CHEM RES, 37: (10) 4090-4098 (1998).

S. Vunnum, V. Natarajan, and S. M. Cramer "IMAC: Self-sharpening of protein-modulator interfaces in frontal chromatography", J. Chromatogr. 818: (1) 31-41 (1998)

K. A. Barnthouse,W. Trompeter, R. Jones, P. Inampudi, R. Rupp and S. M. Cramer, Purification of Recombinant Human Brain-Derived Neurotropic Factor (rHuBDNF) by Cation-Exchange Displacement Chromatography", J. of Biotech. 66: (2-3) 125-136 (1998).

A. A. Shukla, K. A. Barnthouse, S. S. Bae, J. A. Moore and S. M. Cramer, "Structural Characteristics of low molecular weight displacers for cation-exchange chromatography", J. Chromatogr. 814: (1-2) (1998)

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Advances and Perspectives, Vol. 4: High Performance Liquid Chromatography, edited by Csaba Horvath, reviewed for American Scientist (1988).

THESIS SUPERVISION

Masters Thesis

Stuart Gallant: "Neural Network Applications in Chromatography", May 1991.

Regina Mesquita: "Purification of Natural Products by Displacement Chromatography", completed 1992

Partha Burogohain: "Ultrapurification of Isopropyl alcohol", completed 1995.

Khurram Sunasara: "High Temperature Reversed Phase Displacement Chromatography of BDNFcompleted 1997.

Manish Goel: "Hybrid Membrane Processes for Metal Plating Waste Recycling". conferred September 1995.

Vikash Agrawal: "Hybrid Membrane Processes for Metal Plating Waste Recycling". Completed in 1998.

Sanchayita Ghoses: "Modeling of Novel Chromatographic Media", in process.

Doctoral Theses

Guhan Subramanian: "Displacement Chromatography of Biomolecules", degree conferred May 1990.

Michael Phillips: "Optimization and Scale-Up of Displacement Chromatography", degree conferred May 1991.

Dauh-Rung Wu: "Multiphase Enzyme Reactors for Organic Synthesis", conferred December 1991.

Clayton Brooks, III: "On the Characterization of Equilibrium in Ion Exchange Chromatography: Steric Mass Action Ion Exchange", conferred August 1993.

Guhan Jayaraman: "Ion-Exchange Displacement Chromatography of Proteins: Heuristic Approaches to Displacer Design", conferred August 1993.

Joseph Gerstner: "Membrane Chromatography", conferred May 1993.

Young Kim: "Metal Affinity Displacement Chromatography of Protein", conferred May 1993.

Shishir Gadam: "Characterization of Non-Linear Adsorption Properties of Biopolymers", conferred May 1994.

Stuart Gallant: "Control of Optimization of Displacement Systems", conferred, 1995.

Amitava Kundu: "Low Molecular Weight Displacers for Protein Purification in Ion Exchange Systems", conferred July 1996.

Suresh Vunnum, "Non-Linear Protein Separations by Immobilized Metal Affinity Chromatography", conferred, December 1996.

Kristopher Barnthouse, "Purification of Complex Biological Mixtures by High Throughtput Chromatography", expected May 1997.

Abhinav Shukla "Novel Low Molecular Weight Displacers", conferred 1999.

Venkatesh Nataragan "Optimization of Preparative Ion Exchange Chromatography"", conferred 1999.

Samir Sanes "RAMAN and FTIR for evaluation of protein conformation on chromatographic surfaces".", conferred 1999.

Cecilia Mazza "Quantitative Structure Efficacy Relationship Models of Displacement Chromatography", in process.

Khurram Sunsasara "Hydrophobic Displacement Chromatography", in prcess.

Nihal Tugcu "Purification of oligonucleotides by displacement chromatography", in process.

Kaushal Regee "High Throughput Screening of Novel Displacers", in process.

Fang Xia "Development of novel displacers for hydrophobic systems", in process.

Rohit Jindal "Chromatography on a Chip", in process.

ACTIVITIES

Accomplished jazz and classical pianist.